GGCTTCTCGTGGTTCCCAGAGCCCTGCTTAATGGATGGAGACTGGACGAGAACCTGGCTGCTGTGGTTCT
GAACATGGCCCAGAGCCCTGTGTCTGCCGAGGTCATTCACCAGGTGGAAGAGTGTCTTGATGAAGACGAG
AAGGAGATGATGCTCTTCCTGTGTAGAGATGTGACTGAGAACCTGGCTGCACCTAACGTCAGGGACCTCC
TGGATAGCTTAAGTGAGAGAGGCCAGCTCTCTTTTGCTACCTTGGCTGAATTGCTCTACAGAGTGAGGCG
GTTTGACCTTCTCAAGAGGATCTTGAAGACAGACAAAGCAACCGTGGAGGACCACCTGCGCAGAAACCCT
CACCTGGTTTCTGATTATAGGGTCCTGCTGATGGAGATTGGTGAGAGCTTAGATCAGAACGATGTATCCT
CCTTAGTTTTCCTTACAAGGATTACAAGGGATTACACAGGCAGAGGCAAGATAGCCAAGGACAAGAGTTT
CTTGGATCTGGTGATTGAATTGGAGAAACTGAATCTAATTGCTTCAGACCAATTGAATTTGTTAGAAAAA
TGCCTGAAGAACATCCACAGAATAGACTTGAACACAAAGATCCAGAAGTACACCCAGTCCAGCCAAGGAG
CAAGATCAAATATGAATACTCTCCAGGCTTCGCTCCCAAAATTGAGTATCAAGTATAACTCAAGGCTCCA
GAATGGGCGAAGTAAAGAGCCAAGATTTGTGGAATACCGTGACAGTCAAAGAACACTGGTGAAGACATCC
ATCCAGGAATCAGGAGCTTTTTTACCTCCGCACATCCGTGAAGAGACTTACAGGATGCAGAGCAAGCCCC
TAGGAATCTGCTTGATCATTGATTGTATTGGCAACGACACAAAATATCTTCAAGAGACCTTCACTTCCCT
GGGCTATCATATCCAGCTTTTCTTGTTTCCCAAGTCACATGACATAACCCAGATTGTTCGCCGATATGCA
AGTATGGCCCAACATCAAGACTATGACAGCTTTGCATGTGTTCTGGTGAGCCTAGGAGGCTCCCAAAGCA
TGATGGGCAGAGATCAAGTTCACTCAGGGTTCTCCTTGGATCATGTCAAGAACATGTTCACGGGGGACAC
GTGCCCTTCTCTCAGAGGGAAGCCAAAGCTCTTTTTTATTCAGAACTATGAGTCGTTAGGTAGCCAGTTG
GAAGATAGCAGCCTGGAGGTAGATGGGCCATCAATAAAAAATGTGGACTCTAAGCCCCTGCAACCCAGAC
ACTGCACAACTCACCCAGAAGCTGATATCTTTTGGAGCCTGTGCACAGCAGACGTATCTCACTTGGAGAA
GCCCTCCAGCTCATCCTCTGTGTATCTGCAGAAGCTCTCCCAGCAGCTGAAGCAAGGCAGGAGACGCCCA
CTCGTGGACCTCCACGTTGAACTCATGGACAAAGTGTATGCGTGGAACAGTGGTGTTTCGTCTAAGGAGA
AATACAGCCTCAGCCTGCAGCACACTCTGAGGAAGAAACTCATCCTGGCTCCTACGTGAGAACCCCAGAC
CGTTGGTGTTCTTGGTATATCATCCAGGGTGGCGGCTTGGAGCAGAGCTTGGCGGTTACGGCTGCTTCTG
GCTGCTTCTGGCTCTGCCGTGAGTCCTGGCCTAGGGTTCTCCTGTGCACAGGCATGAGCCGTAACCCTGT
GCCTGGGAAACGTCTCACTCCCGCCGCCGTGCCTTTACCTCTCTAAACTTCCCTACTTACATTCCTTAGT
CGGATGTTTTGCCAGAGTGTGGAGAACAGTAAGACATAAACCTATTGTTTGTTTGTTTTTTTGGGGGGGA
GGTTATCTACCAAGTTATACCAAGTTATTGTATGGGTGTATAGTGTATAGTGGTTCAAGATTCTGAATGT
AACTTGAGACTTACCTGAGTTTGTCATGCGACTGGGTAAATTGTTTCTATGGCACATCTAATCATTTAAT
AAGTAATTACCTCATTAAGTACCCATTGCTTCAGGACTTTCACATTGGCCACCAATTTCTGTGACCCAGC
TCCACATTTATATTCTCTTTCGGCAAAACCAAATTTCATTATGTCTGTTTAATATCTACAGTCTAATGCT
TTGTAAGACATCTAGATAGGAAAAATAGTTACCCATGAGCACAGGAGGGCTGGCCTGACCCTCACCAGCTG
TGCAGTGGCTTCGGTGAAAGGAGAATGAGCCCTACTCCTTGAAAGGTTGTAGTGCTTGGGAGAGCAGTCT
GTACCTTGCCTGGGCAGCACAGTAGAGCCAGCCCCAAGAACACAACAGTGAGTGGGGGAGCTTGCCCTGG
TTGGCTCAGGATCAGGAAACAGGAGGGATGACCAACTTGGGGCTTTGAGGTGGCCCACCCCAGCATCCAT
ATCATCTGTGAACTGCCAGAGCCTGTGAAGGGGCGGGTCCTGTAGAACTAAGGCTGCAGGATCTCCATGA
CACAGGGCAACAACAGGGTATCTGAGAAGGGTCCCCGTGAGGGTCCAGTATTTATAGTGCACCAGAAGCC
AGAGGCCTCGGATCAGACAATGACCCATTGCACTGAGTAAAGATGTAAGTGAATGAGTGAAGATGTGTGG
GCACACGGAAATACTGAGGGACACACACAAGCTTTTATGGAGATGTTTGTTTGTTTGTTTGTTTGTTTTT
TGTTTCTTTGGCAGGAACAGATTGCAAGGGCAGAGAGTAGATAAGGAAGCTGGAGACATGAGTGGGGTTG
GGTGCATGATATAGAATTCACAAAGAAAAAAAAAAAAAA (SEQ ID NO:1)

MAQSPVSAEVIHQVEECLDEDEKEMMLFLCRDVTENLAAPNVRDLLDSLSERGQLSFATLAELLYRVRRFDLLKRILKTDKATVED
HLRRNPHLVSDYRVLLMEIGESLDQNDVSSLVFLTRITRDYTGRGKIAKDKSFLDLVIELEKLNLIASDQLNLLEKCLKNIHRIDL
NTKIQKYTQSSQGARSNMNTLQASLPKLSIKYNSRLQNGRSKEPRFVEYRDSQRTLVKTSIQESGAFLPPHIREETYRMQSKPLGI
CLIIDCIGNDTKYLQETFTSLGYHIQLFLFPKSHDITQIVRRYASMAQHQDYDSFACVLVSLGGSQSMMGRDQVHSGFSLDHVKNM
FTGDTCPSLRGKPKLFFIQNYESLGSQLEDSSLEVDGPSIKNVDSKPLQPRHCTTHPEADIFWSLCTADVSHLEKPSSSSSVYLQK
LSQQLKQGRRRPLVDLHVELMDKVYAWNSGVSSKEKYSLSLQHTLRKKLILAPT
(SEQ ID NO:2)

FIGURE 1

<u>underlined</u> = deleted in targeting construct

[ ] = sequence flanking Neo insert in targeting construct

[GGCTTCTCGTGGTTCCCAGAGCCCTGCTTAATGGATGGAGACTGGACGAGAACCTGGCTG
CTGTGGTTCTGAACATGGCCCAGAG] **CCCTGTGTCTGCCGAGGTCATTCACCAGGTGGAAG**
AGTGTCTTGATGAAGACGAGAAGGAGATGATGCTCTTCCTGTGTAGAGATGTGACTGAGA
ACCTGGCTGCACCTAACGTCAGGGACCTCCTGGATAGCTTAAGTGAGAGAGGCCAGCTCT
CTTTTGCTAC [CTTGGCTGAATTGCTCTACAGAGTGAGGCGGTTTGACCTTCTCAAGAGGA
TCTTGAAGACAGACAAAGCAACCGTGGAGGACCACCTGCGCAGAAACCCTCACCTGGTTT
CTGATTATAG] GGTCCTGCTGATGGAGATTGGTGAGAGCTTAGATCAGAACGATGTATCCT
CCTTAGTTTTCCTTACAAGGATTACAAGGGATTACACAGGCAGAGGCAAGATAGCCAAGG
ACAAGAGTTTCTTGGATCTGGTGATTGAATTGGAGAAACTGAATCTAATTGCTTCAGACC
AATTGAATTTGTTAGAAAAATGCCTGAAGAACATCCACAGAATAGACTTGAACACAAAGA
TCCAGAAGTACACCCAGTCCAGCCAAGGAGCAAGATCAAATATGAATACTCTCCAGGCTT
CGCTCCCAAAATTGAGTATCAAGTATAACTCAAGGCTCCAGAATGGGCGAAGTAAAGAGC
CAAGATTTGTGGAATACCGTGACAGTCAAAGAACACTGGTGAAGACATCCATCCAGGAAT
CAGGAGCTTTTTTACCTCCGCACATCCGTGAAGAGACTTACAGGATGCAGAGCAAGCCCC
TAGGAATCTGCTTGATCATTGATTGTATTGGCAACGACACAAAATATCTTCAAGAGACCT
TCACTTCCCTGGGCTATCATATCCAGCTTTTCTTGTTTCCCAAGTCACATGACATAACCC
AGATTGTTCGCCGATATGCAAGTATGGCCCAACATCAAGACTATGACAGCTTTGCATGTG
TTCTGGTGAGCCTAGGAGGCTCCCAAAGCATGATGGGCAGAGATCAAGTTCACTCAGGGT
TCTCCTTGGATCATGTCAAGAACATGTTCACGGGGGACACGTGCCCTTCTCTCAGAGGGA
AGCCAAAGCTCTTTTTTATTCAGAACTATGAGTCGTTAGGTAGCCAGTTGGAAGATAGCA
GCCTGGAGGTAGATGGGCCATCAATAAAAAATGTGGACTCTAAGCCCCTGCAACCCAGAC
ACTGCACAACTCACCCAGAAGCTGATATCTTTTGGAGCCTGTGCACAGCAGACGTATCTC
ACTTGGAGAAGCCCTCCAGCTCATCCTCTGTGTATCTGCAGAAGCTCTCCCAGCAGCTGA
AGCAAGGCAGGAGACGCCCACTCGTGGACCTCCACGTTGAACTCATGGACAAAGTGTATG
CGTGGAACAGTGGTGTTTCGTCTAAGGAGAAATACAGCCTCAGCCTGCAGCACACTCTGA
GGAAGAAACTCATCCTGGCTCCTACGTGAGAACCCCAGACCGTTGGTGTTCTTGGTATAT
CATCCAGGGTGGCGGCTTGGAGCAGAGCTTGGCGGTTACGGCTGCTTCTGGCTGCTTCTG
GCTCTGCCGTGAGTCCTGGCCTAGGGTTCTCCTGTGCACAGGCATGAGCCGTAACCCTGT
GCCTGGGAAACGTCTCACTCCCGCCGCCGTGCCTTTACCTCTCTAAACTTCCCTACTTAC
ATTCCTTAGTCGGATGTTTTGCCAGAGTGTGGAGAACAGTAAGACATAAACCTATTGTTT
GTTTGTTTTTTTGGGGGGGAGGTTATCTACCAAGTTATACCAAGTTATTGTATGGGTGTA
TAGTGTATAGTGGTTCAAGATTCTGAATGTAACTTGAGACTTACCTGAGTTTGTCATGCG
ACTGGGTAAATTGTTTCTATGGCACATCTAATCATTTAATAAGTAATTACCTCATTAAGT
ACCCATTGCTTCAGGACTTTCACATTGGCCACCAATTTCTGTGACCCAGCTCCACATTTA
TATTCTCTTTCGGCAAAACCAAATTTCATTATGTCTGTTTAATATCTACAGTCTAATGCT
TTGTAAGACATCTAGATAGGAAAAATAGTTACCCATGAGCACAGGAGGGCTGGCCTGACC
CTCACCAGCTGTGCAGTGGCTTCGGTGAAAGGAGAATGAGCCCTACTCCTTGAAAGGTTG
TAGTGCTTGGGAGAGCAGTCTGTACCTTGCCTGGGCAGCACAGTAGAGCCAGCCCCAAGA
ACACAACAGTGAGTGGGGGAGCTTGCCCTGGTTGGCTCAGGATCAGGAAACAGGAGGGAT
GACCAACTTGGGGCTTTGAGGTGGCCCACCCCAGCATCCATATCATCTGTGAACTGCCAG
AGCCTGTGAAGGGGCGGGTCCTGTAGAACTAAGGCTGCAGGATCTCCATGACACAGGGCA
ACAACAGGGTATCTGAGAAGGGTCCCCGTGAGGGTCCAGTATTTATAGTGCACCAGAAGC
CAGAGGCCTCGGATCAGACAATGACCCATTGCACTGAGTAAAGATGTAAGTGAATGAGTG
AAGATGTGTGGGCACACGGAAATACTGAGGGACACACACAAGCTTTTATGGAGATGTTTG
TTTGTTTGTTTGTTTGTTTTTTGTTTCTTTGGCAGGAACAGATTGCAAGGGCAGAGAGTA
GATAAGGAAGCTGGAGACATGAGTGGGGTTGGGTGCATGATATAGAATTCACAAAGAAAA
AAAAAAAAAA

FIGURE 2A

Gene Sequence Structure *

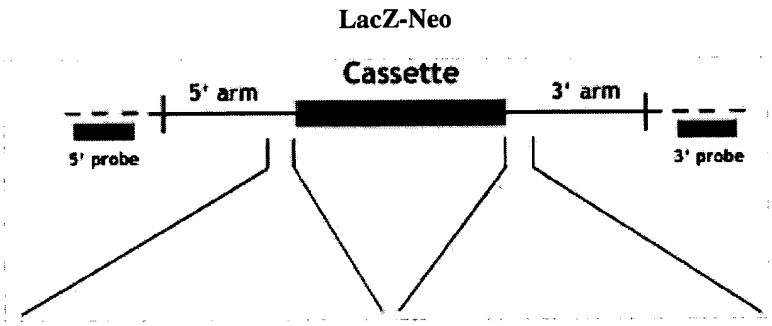
86 bp Sequence Deleted 250 bp

Size of full-length cDNA: 2770 bp

Targeting Vector* (genomic sequence)

Construct Number: 3547

Arm Length:
5': 0.3 kb
3': 4.8 kb



5'>CCTGTGCTTTGACTCTCAAGC CTAAGTGTTTTGATAAGAGGATTC TCTTTCACCACAGAGTGTCTCTAT TGCAAGAACTCTGAGAGAAATGAA GAGAGTCCTCAGCAATGATGTTGG CTTCTCGTGGTTCCCAGAGCCCTG CTTAATGGATGGAGACTGGACGAG AACCTGGCTGCTGTGGTTCTGAAC ATGGCCCAGAG<3' (SEQ ID NO:3)	5'>CTTGGCTGAATTGCTCTACAG AGTGAGGCGGTTTGACCTTCTCAA GAGGATCTTGAAGACAGACAAAGC AACCGTGGAGGACCACCTGCGCAG AAACCCTCACCTGGTTTCTGATTA TAGGTAAGTCATCCCCTGGGGGAG GGGAGAGGGAGTCTAGATGGTTAG GGCAGTGAGAAGACCCCATTGCTT CCTCTTCTCTC<3' (SEQ ID NO:4)

Targeting Vector
Endogenous Locus

* Not drawn to scale

FIGURE 2B

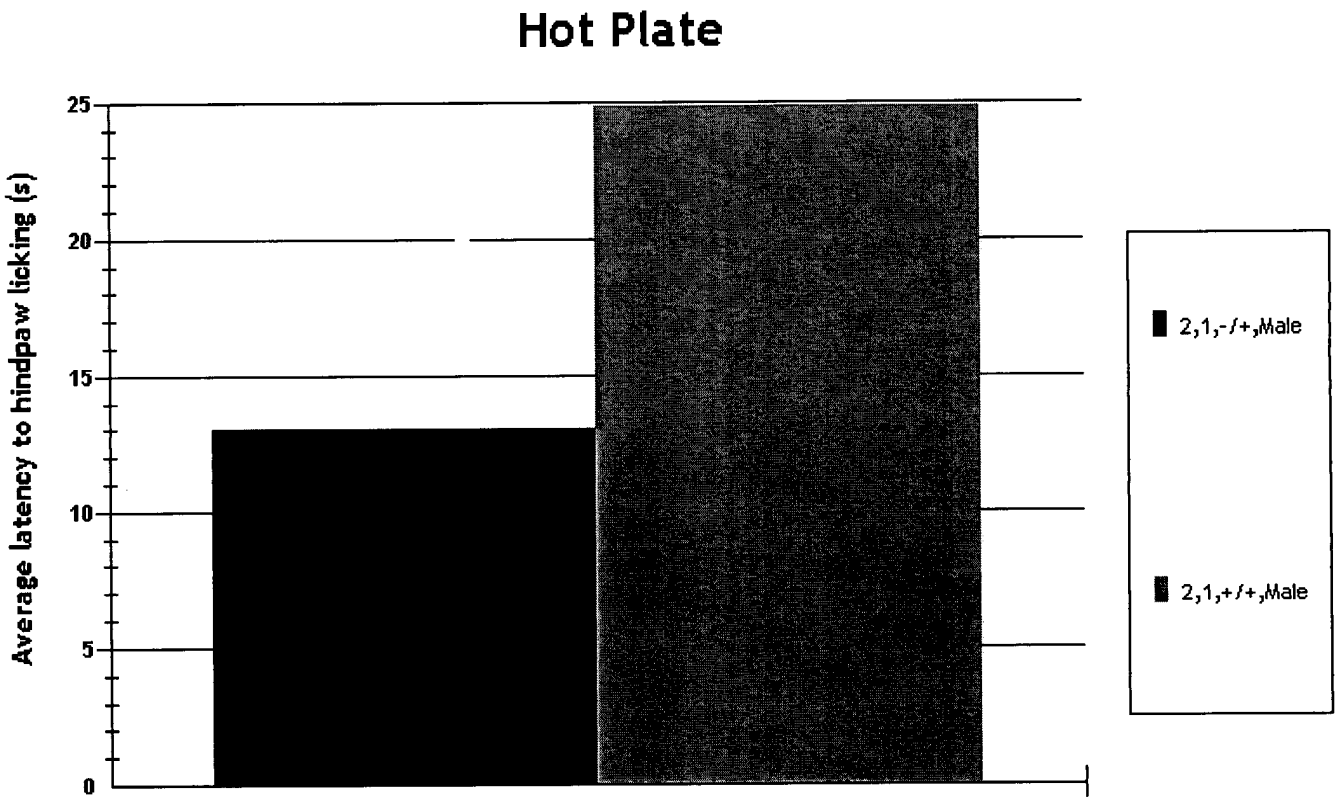

Hot Plate
Average latency to hindpaw licking (s)
25
20
15
10
5
0
2,1,-/+,Male
2,1,+/+,Male

FIGURE 3

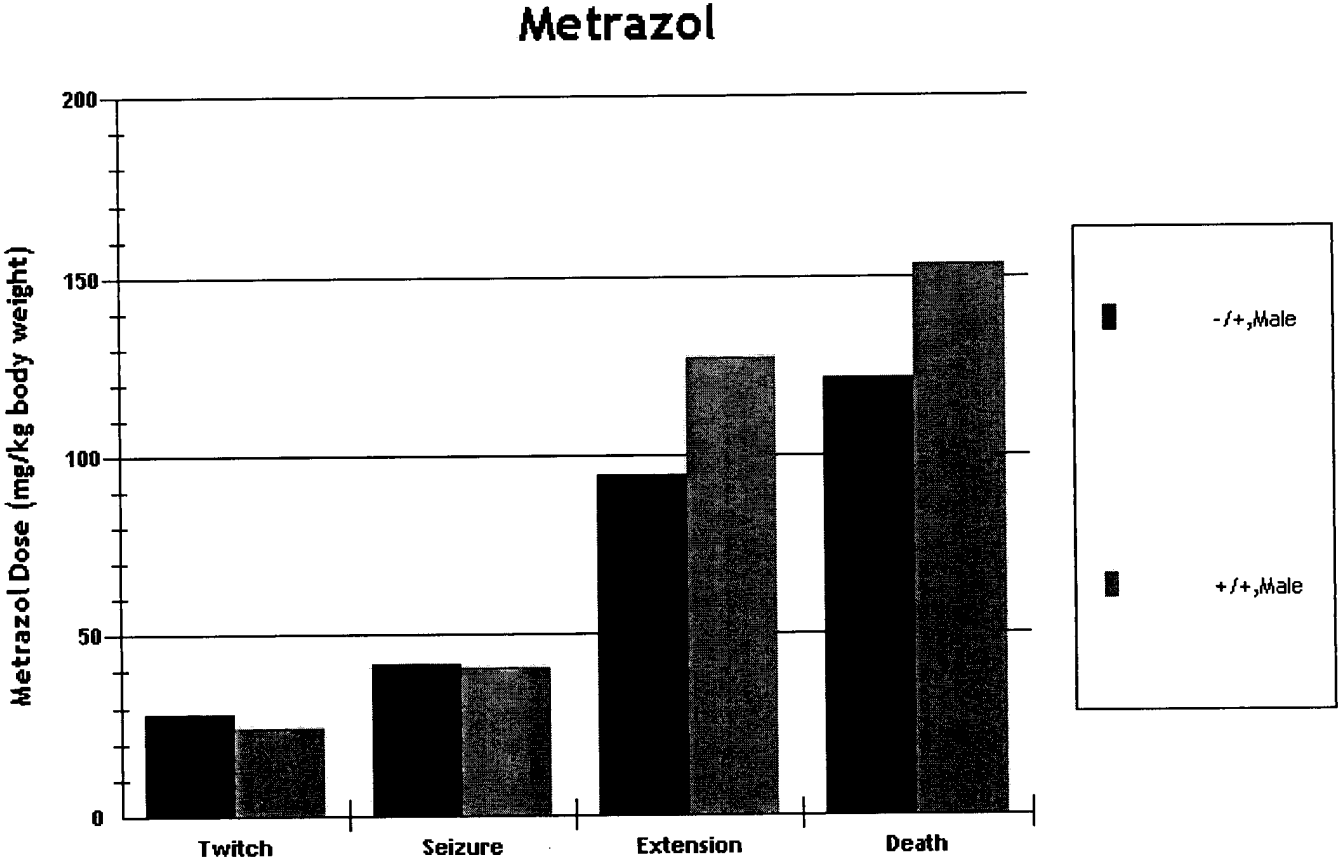

Metrazol
Metrazol Dose (mg/kg body weight)
200
150
100
50
0
Twitch
Seizure
Extension
Death
-/+,Male
+/+,Male

FIGURE 4